

Electrochemistry of Spin Adduct

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A spin trapping method for the detection of short-lived free radicals in solution using electron spin resonance (ESR) spectroscopy has been developed and widely used since the 1960s. Compared with other ESR methods such as rapid-mixing flow cell systems, spin trapping has the advantages that only a small amount of sample volume is needed, independent of its short spin relaxation times at room or physiological temperature. Especially in recent years, with the development of a new spin trapping reagent and low-frequency ESR spectroscopy, the spin trapping method has been a very important technology for imaging of reactions *in vivo*.

Though many advances in ESR have been achieved over these decades, the ESR apparatus is not inexpensive and analyses of its spectra require extensive knowledge and experience in quantum chemistry. Furthermore, in the reactions of spin adduct generation from radicals and trapping reagents, the area of most concern for a researcher is whether ESR signals can be obtained or not. We cannot say that the reaction mechanism involved has been sufficiently elucidated.

The reason we can detect spin adducts by ESR is that they have a hindered paramagnetic nitroxyl group ($>\text{N-O}\cdot$) after spin trapping. From the early period in the spin trapping method, though the detection of spin adducts from electrogenerated radicals was reported, the obtained adducts were regarded as electroinactive species. Since the 1980s, 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO) derivatives, which also have a hindered paramagnetic nitroxyl group, have undergone a renaissance through their redox property for catalytic applications with organic compounds. In that case, the electroactivity of TEMPO derivatives was widely recognized, and they were used as electron mediators and chemically modified electrodes. Aside from the above description, there have been attempts to employ electrochemical detectors in high-performance liquid chromatography for the detection of spin adducts in biological science.¹ However, they were not able to achieve sufficient quantification because the electrochemical property of the spin adducts had not been clarified.

In this study, we report the redox property of spin adducts which are generated by the reaction of a radical and spin trapping reagents in solutions. In order to investigate them in simpler systems at first, the superoxide ion (O_2^-) which is generated from potassium superoxide solubilized by crown ether into dimethyl sulfoxide was used as a radical source, and 5,5-dimethyl-1-pyrroline-N-oxide or *N-tert*-butyl- α -phenylnitrone as typical spin trapping reagents of nitroxide and nitroso compounds were utilized. Ordinarily, ESR signal of O_2^- could not be observed at room temperature because of its short spin relaxation times, so the spin trapping method was adopted for its detection.

Potassium superoxide (KO_2 , Aldrich), dibenzo-18-crown-6-ether (DB18C6, Wako Pure Chemicals), 5,5-dimethyl-1-pyrroline N-oxide (DMPO, Tokyo Kasei), *N-tert*-butyl- α -phenyl-nitrone (BPN, Tokyo Kasei), dimethylsulfoxide (DMSO, Wako Pure Chemicals), and

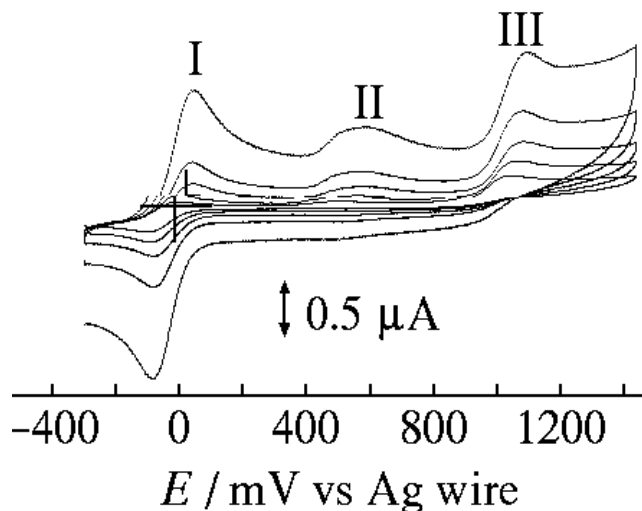
acetonitrile (ACN, Wako Pure Chemicals) were used as received. ESR spectra were measured with a JES-RS-RE2X spectrometer. A computer-controlled potentiostat (BAS 100B/W) was employed for a conventional three-electrode electrochemical measurement with a glassy carbon working electrode (1mm diameter), a Pt wire auxiliary electrode and an Ag wire quasi-reference electrode. All measurements were performed at room temperature.

The spin adduct from O_2^- which is generated from KO_2 solubilized by DB18C6, and DMPO in DMSO shows an ESR spectrum which is similar to that reported.² However, in the case of ACN as a solvent, the ESR signal was not well-defined and essentially disappeared after a few minutes. The cyclic voltammogram of the spin adduct from O_2^- and DMPO in DMSO showed a quasi-reversible redox peak at 0 mV (peak I) and irreversible oxidation peaks at 500 and 100 mV (peaks II and III). Peak I is attributed to the oxidation/reduction of dissolved O_2^- which has not yet reacted with DMPO, indicating the sluggishness and low yield of the reaction of O_2^- and DMPO ($\sim 10 \text{ M}^{-1}\text{s}^{-1}$). It is of interest in obtaining an ESR signal in spite of the low reactivity.

On the other hand, the spin adduct from O_2^- and BPN in DMSO shows an ESR spectrum which has triplet splitting. The cyclic voltammogram of the spin adduct from O_2^- and BPN showed only the redox peak I and the irreversible oxidation peak III, suggesting that the oxidation at peak III corresponded to the electrode reaction of the nitroxyl group in the spin adduct.

References

1. R. A. Floyd, C. A. Lewis, and P. K. Wong, in *Methods in Enzymology*, (L. Packer, Ed.) **105**, p231, Academic Press, San Diego, (1984).
2. T. Ozawa and A. Hanaki, *Chem. Pharm. Bull.*, **26**, 2572 (1978).



Sweep rate: 200, 100, 50, 20, 10 mVs^{-1}